FOUR-STRANDED DNA AS DETERMINED BY ELECTRON MICROSCOPY

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ABSTRACT

Pneumococcus DNA, of weight-average molecular weight 1.6 million by light scattering, had a weight-average length of 4300 A by electron microscopy. Thus, the average mass per unit length was 370 molecular-weight units per A, or approximately two times that expected (208) for a Watson-Crick double helix. This corresponds to an average of 3.6 strands per molecule, which is close to that obtained by other methods. Morphologically, all the particles in the micrographs were relatively stiff, and had a cross-sectional height of 20 to 30 A. Some divided into two stiff branches of the same height, apparently double helical. Where the branches combined into one (minimally four-stranded) structure they apparently lay side by side in close association.

INTRODUCTION

It has been the aim of our recent investigations to elucidate some of the steps in the replication of DNA (Cavalieri, Rosenberg, and Deutsch, 1959; Cavalieri, Deutsch, and Rosenberg, 1961; Cavalieri and Rosenberg, 1961 *a*, *b*, *c*; Cavalieri, Finston, and Rosenberg, 1961). The experimental approach has involved (1) examination of the molecular weights of DNA from various sources, (2) demonstration of the existence of two classes of DNA molecules, (3) the counting of the number of polynucleotide strands per molecule of each type, and (4) a study of the changes in the number of strands per DNA molecule during the replication cycle. Strand counting was based on the kinetics of degradation by both deoxyribonuclease II and x-rays. A variety of experimental techniques have been employed in these investigations, and have produced a consistent set of results. The two molecular classes of DNA, which have been called unitary and biunial, were found to be composed of double helices and pairs of double helices, respectively. Unitary molecules have been identified with the entities which are conserved during the replication of DNA.

The present investigation represents still another approach to the determination of the number of strands in the DNA molecule. Its experimental basis, electron microscopy, is entirely different from that of the previous investigations. In addition to determining statistical properties of the DNA, this technique permits observation of certain morphological features of individual molecules (Hall, 1956 *a*, *b*; Hall and Litt, 1958). In combination with light scattering, it has enabled us to determine the average mass per unit length of *pneumococcus* DNA.

EXPERIMENTAL

The DNA used in this study was obtained from *Diplococcus pneumoniae* and is one of the samples, designated LC-II-540, used in previous studies dealing with disaggregation and x-ray kinetics (Cavalieri, Deutsch, and Rosenberg, 1961, and Cavalieri,

Finston, and Rosenberg, 1961). Its weight-average molecular weight is 1.6 million and its radius of gyration is 1100 A, as determined by light scattering. It was prepared by a modification of the sodium acetate method of Meyers and Spizizen (1954). The bacteria were centrifuged and resuspended in 5 ml of 0.1 M EDTA–0.1 M sodium chloride, pH 7.5. The mixture was warmed to 60° to help denature intracellular enzymes before lysis; then 50 ml more of solvent, which also contained 10 ml of 5 per cent deoxycholate, were added. Fifty ml of saturated sodium acetate were added to the resulting gel and the mixture was gently stirred with a magnetic bar at 60° for 15 minutes. From this point the directions of Meyers and Spizizen were followed.

Electron micrographs were obtained by procedures described previously (Hall, 1956 *a*; Hall and Litt, 1958). The concentration of the sprayed solution was sufficiently low (0.012 mg/ml) that particles were well separated in the fields, the mean density being less than 2 particles per square micron. We calculate that in solution there are about 4 particles per cubic micron. There was, therefore, slight chance for aggregation. (In order to show as many particles as possible, Figs. 1 and 2 were chosen from areas of abnormally high density.) It is well known that DNA molecules which are many microns long are broken by shear gradients and spray techniques (Cavalieri and Rosenberg, 1959), but we believe these effects are not significant here since we are dealing with particles whose lengths are mostly less than 1 micron while the droplet diameters are about 5 microns. The volume of droplets can be measured from counts of standard particle suspensions sprayed on collodion, but droplets sprayed on mica spread over such large areas that an entire drop pattern cannot usually be contained in a field.

RESULTS

The lengths of 587 particles in the micrographs were measured. Two types of particles, branched and unbranched, could be distinguished. The majority were relatively stiff, separate, unbranched rods, as shown in Fig. 1. Most of these particles resembled those we have previously interpreted to be Watson-Crick double helices. The two particles designated by arrows show a thickening which suggests partial separation of two elements lying side by side, but for the most part if is not possible to say whether these rods are one double helix or two lying close together. A smaller number of particles (66) were branched at one or both ends, as indicated by arrows in Fig. 2. We do not believe that the branches could be interpreted as partially separated single strands of a double helix, since they are too thick and too rigid. Single strands would assume a random coil configuration, like denatured DNA, appearing as flattened patches (Hall and Litt, 1958).

All the particles, both branched and unbranched, have approximately the same height, (20 to 30 A), as calculated from shadow lengths. There is no consistent or significant increase in shadow length where the two branches come together into one structure (*i.e.*, their height perpendicular to the grid surface is generally unchanged by their association). This indicates that the two elements probably lie side by side in close association rather than twisted into a supercoil about one another.

In addition to the two classes of particle just

DNA from *Diplococcus pneumoniae*, shadowed with platinum on a mica substrate. Magnification, 100,000.

FIGURE 1

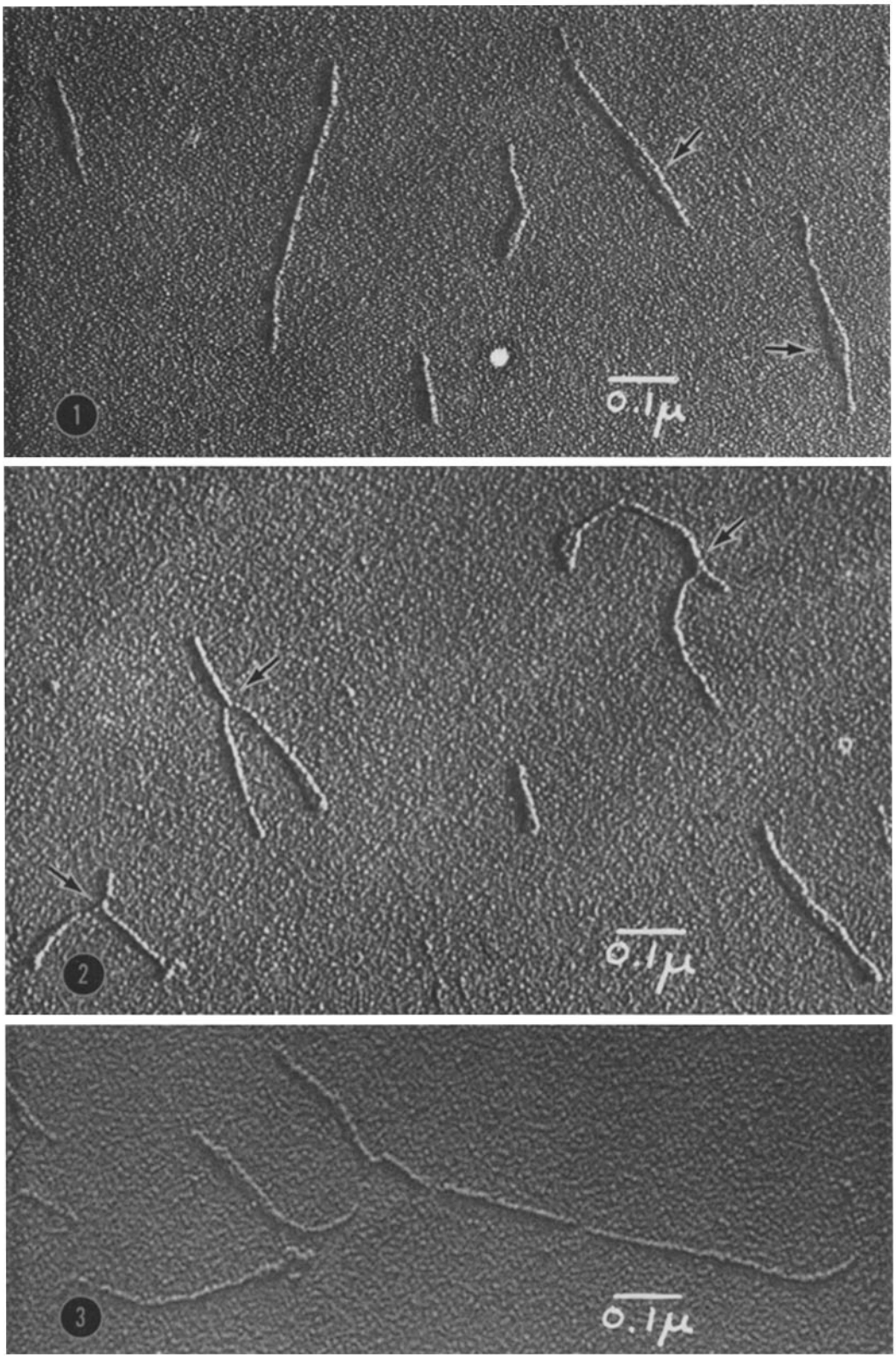
Typical stiff, rod-shaped particles of DNA. Indicated by the arrows are particles showing a thickening which could be interpreted as a partial separation of two elements lying side by side.

FIGURE 2

Indicated by the arrows are three DNA particles showing branching.

FIGURE 3

Several particles of DNA associated in a complex aggregate, excluded from the statistical analysis.


1
0.1μ
2
0.1μ
3
0.1μ

described there was a small amount of material (10 particles) involved in more complex association, as exemplified in Fig. 3. These aggregates, which may or may not have been artifacts produced on drying, have been omitted from the numerical analysis. Since they are few we judge that their omission does not appreciably alter the calculated average number of strands per particle.

Fig. 4 shows the total length of branched and unbranched DNA occurring at each length interval. When the two branches were of unequal length an average was used in the tabulation. If the mass per unit length is the same for both kinds of particle, the ordinates are proportional to the mass of DNA at the respective lengths. If one were to assume that the branched particles have double the mass per unit length of the unbranched, the cross-hatched regions would have to be doubled in height to show the relative masses at the respective lengths.

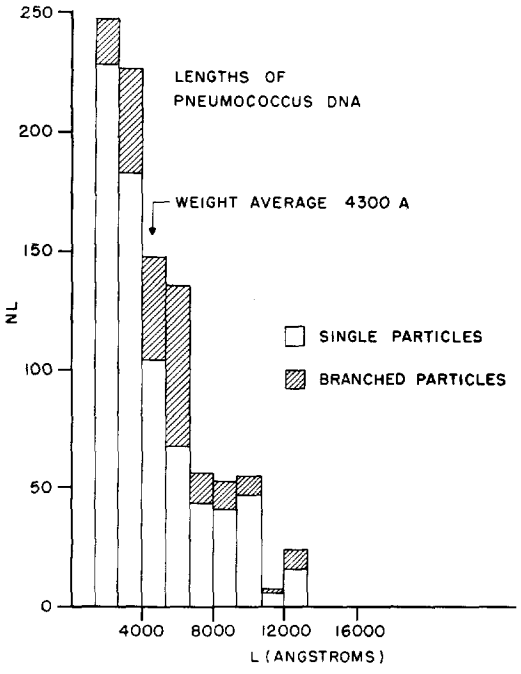


FIGURE 4

The distribution of mass in various particle length categories, assuming that both branched (cross-hatched) and unbranched particles have the same mass per unit length; the mass is then proportional to NL for the given length category, where L is a given particle length and N is the number of particles having that length.

TABLE I

	Number of particles counted	Number average length $\frac{\sum_i N_i L_i}{\sum_i N_i}$	Weight average length $\frac{\sum_i N_i L_i^2}{\sum_i N_i L_i}$
Unbranched	521	2568A	4220A
Branched	66	4300	4650
Both combined	587	2740	4300

The number-average and weight-average lengths computed as indicated (Hall and Doty, 1958) are summarized in Table I. Because the weight averages for branched and unbranched particles are closely similar, the calculated mass per unit length of the particles is relatively insensitive to morphological considerations. (The unbranched particles do have a significantly shorter number-average length due to the fact that in this class there is a large number of short particles which, however, have relatively small effect on the weight average.) Thus if one divides the weight-average molecular weight from light scattering (1.6×10^6) by the weight-average length, one obtains 370 molecular-weight units per A or approximately twice the value (208) for a Watson-Crick double helix (Watson and Crick, 1953; Langridge *et al.*, 1960). This is equivalent to 3.6 strands per molecule.

CONCLUSIONS

On the basis of morphological evidence, that is, branching, we conclude that there is a significant number of four-stranded particles. If the branched particles are four-stranded throughout their length and, therefore, double the weight per unit length of the unbranched particles assumed to be two-stranded, this would mean that at least 30 per cent of the material by weight is in the four-stranded category. (Relative areas in Fig. 4.) On the basis of mass per unit length obtained by combining the weight-average length and molecular weight one would have to conclude that most of the unbranched particles (*i.e.*, all but 10 per cent of the total mass) must be four-stranded as well. Of course, if the branched particles had more than four strands, a higher percentage of the unbranched particles would

have to be two-stranded so that the average number of strands would remain close to four. There is no reason to assume, however, that such a fundamental difference does exist between branched and unbranched particles, especially since branched particles have nearly the same weighted length as the rest of the population.

Electron microscopy in conjunction with molecular weight data strongly suggest that *pneumococcus* DNA has an average of close to four strands per molecule; these appear to be organized as a pair of loosely linked double helices. The same conclusions have previously been reached by different means (Cavalieri and Rosenberg, 1961 *a*, *b*, *c*). In addition, there is some evidence that the molecules are more or less uniformly four-stranded. A morphological feature of the utmost significance for the mechanism of DNA replication has also come to light: the two double helices of the biunial molecule lie side by side rather than intertwined. This suggests that when the double helices, which Cavalieri and Rosenberg have concluded are the conserved units, replicate, they form new partners by some mechanism which permits the transfer of information (without separation of the strands of the double helix) to an adjacent rather than concentric structure. A mechanism consistent with these requirements has been proposed (Cavalieri and Rosenberg, 1961 *d*).

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REFERENCES

CAVALIERI, L. F., and ROSENBERG, B. H., 1959, *J. Am. Chem. Soc.*, **80**, 5136.

CAVALIERI, L. F., DEUTSCH, J. F., and ROSENBERG, B. H., 1961, *Biophysic. J.*, **1**, 301.

CAVALIERI, L. F., FINSTON, R., and ROSENBERG, B. H., 1961, *Nature*, **89**, 833.

CAVALIERI, L. F., and ROSENBERG, B. H., 1961 *a*, *Biophysic. J.*, **1**, 317.

CAVALIERI, L. F., and ROSENBERG, B. H., 1961 *b*, *Biophysic. J.*, **1**, 323.

CAVALIERI, L. F., and ROSENBERG, B. H., 1961 *c*, *Biophysic. J.*, **1**, 337.

CAVALIERI, L. F., and ROSENBERG, B. H., 1961 *d*, to be published.

CAVALIERI, L. F., ROSENBERG, B. H., and DEUTSCH, J. F., 1959, *Biochem. and Biophys. Res. Comm.*, **1**, 124.

HALL, C. E., 1956 *a*, *J. Biophysic. and Biochem. Cytol.*, **2**, 625.

HALL, C. E., 1956 *b*, *Proc. Nat. Acad. Sc.*, **42**, 801.

HALL, C. E., and DOTY, P., 1958, *J. Am. Chem. Soc.*, **80**, 1269.

HALL, C. E., and LITT, M., 1958, *J. Biophysic. and Biochem. Cytol.*, **4**, 1.

LANGRIDGE, R., MARVIN, D. A., SEEDS, W. E., WILSON, H. R., HOOPER, C. W., WILKINS, M. H. F., and HAMILTON, L. D., 1960, *J. Molec. Biol.*, **2**, 19.

MEYERS, V. L., and SPIZIZEN, J., 1954, *J. Biol. Chem.*, **210**, 877.

WATSON, J. D., and CRICK, F. H. C., 1953, *Nature*, **171**, 737.